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IMPAIRED REGULATION OF BETA$_2$-ADRENERGIC RECEPTOR DENSITY IN MONONUCLEAR CELLS DURING CHRONIC RENAL FAILURE

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Abstract—Previous investigations have suggested that beta-adrenoceptor-mediated responses were decreased in uremia. To evaluate this phenomenon further, beta$_2$-receptor density in mononuclear cells, plasma catecholamines and plasma parathyroid hormone were studied in two groups of normotensive patients: group U, twenty-five chronic uremic patients with end-stage renal failure; group C, twenty-eight control subjects. Each group was divided into three age and sex-matched subgroups.

Beta$_2$-receptor density was determined using(−)^{125} iodocyanopindolol. Despite a significant increase in plasma epinephrine in the group of uremic patients, there was a significant increase in beta$_2$-adrenoceptor density. On the other hand the uremic state did not influence (−)^{125} iodocyanopindolol binding affinity and plasma norepinephrine. Parathyroid hormone, as expected, was significantly elevated in all the uremic subgroups.

It can be concluded that the uremic state is associated with an unexpected upregulation of beta$_2$-receptor density in mononuclear cells. The role of an endogenous beta-blocking substance is suggested.

Abnormalities in sympathetic nervous system function have been reported both in uremic patients and in animals with experimental uremia, [1, 2]. Uremia is characterized by a decrease in beta-adrenoceptor-mediated responsiveness [3–5]. This desensitization could be explained by a variation of beta-receptor number or affinity.

Radioligand binding assay have led to the discovery that the number of beta$_2$-receptors on the cell surface was regulated by a wide variety of circumstances, including therapeutic interventions and disease [6].

This study was performed to assess the alteration of beta receptors in mononuclear cells (MNC) during end stage chronic renal failure using a radioligand (−)^{125} iodocyanopindolol ((−)^{125} ICYP), with a very high specific radioactivity (≥ 2000 Ci/mmol). The beta$_2$-adrenergic receptor in MNC is a convenient model to study [7–9], since MNC are easily available in relatively purified form. Although the number of beta$_2$-receptors reportedly varies according to the different human lymphocyte subpopulations [10], there is no difference in circulating lymphocyte T subpopulations between hemodialyzed and control subjects [11, 12].

(−)^{125}ICYP is characterized by its specificity for the beta receptor together with a high affinity constant [9] and a lack of selectivity for either beta$_1$ or beta$_2$ subtypes. Its binding parameters, concentration of binding sites per mg of protein (B_{max}), and the corresponding dissociation constant (K_d) were measured after incubation with MNC homogenates. Because of the interaction between beta-adrenoceptors and beta-adrenergic stimulation [13], plasma catecholamines were determined simultaneously.

MATERIALS AND METHODS

We compared MNC beta$_2$-adrenoceptors in uremic and control subjects using a radioligand binding technique. At the same time, plasma epinephrine (E), plasma norepinephrine (NE) and plasma parathyroid hormone (PTH) were determined. All the subjects gave written informed consent.

Patients

Twenty-five normotensive (diastolic blood pressure ≤ 90 mmHg) chronic uremic patients (group U) treated in the same department for varying lengths of time (extreme: 6–114 months) were investigated. The clinical characteristics are listed in Table 1: twenty-two underwent stable and regular haemodialysis, two or three times weekly, and three were treated by continuous ambulatory peritoneal dialysis. The patients were age-matched in their subgroups: group U_1 (mean age: 27.3 ± 1.6 yr, mean blood pressure: 128.1 ± 5.4/74.1 ± 4.9 mmHg); group U_2 (mean age: 57.5 ± 4.7 yr, mean blood pressure: 131.4 ± 3.2/76.3 ± 3.0 mmHg); group U_3 (mean age: 71.0 ± 0.9 yr, mean blood pressure: 125 ± 4.3/70.2 ± 3.7 mmHg). None of the uremic patients had cardiovascular disease as detected by physical examination and echocardiography. Blood pressure was taken as the average of at least three separate measurements in supine position. None had diabetes mellitus. All subjects were within 10% of their ideal body weight (Metropolitan Life Insurance Company Table, 1959). The patients were taking only phosphate-binding agents and vitamin D. They were on normal diet except for moderate salt and fluid restriction.

Table 1. Clinical characteristics of the uremic patients

Group subjects	Age (years)	SBP (mmHg)	DBP (mmHg)	Duration of treatment (months)	Treatment	Sex
U_1						
BLI. N	22	132	82	72	H	F
LUC. B.	24	110	61	38	H	F
CHE. C.	26	116	84	60	H	F
SER. J.	31	113	60	40	H	F
RAB. P.	29	140	71	86	H	M
MAR. P.	32	138	87	94	H	M
Mean	27.3	128.1	74.1	65		
± S.E.M.	1.6	5.4	4.9	9.5		
U_2						
VER. L.	45	140	78	58	H	M
HER. M.	52	140	90	50	H	M
RET. L.	54	132	66	114	H	F
LER. E.	55	120	72	26	H	M
RON. P.	59	128	68	98	H	M
BOU. J.	61	108	58	87	H	M
PER. A.	62	138	84	6	H	M
JOC. J.	62	136	90	34	H	M
LES. L.	62	144	82	82	H	F
GOY. F.	64	136	78	6	H	F
PAC. R.	57	124	74	88	H	M
Mean	57.5	131.4	76.3	59		
± S.E.M.	4.7	3.2	3.0	11.3		
U_3						
CAP. L.	71	140	90	6	H	F
BER. M.	72	110	68	101	H	F
DRO. R.	72	136	80	18	H	M
SEN. A.	74	114	64	38	H	F
HEL. P.	74	136	74	69	H	M
REV. D.	66	114	62	16	APD	F
NOU. MJ.	68	118	60	34	APD	F
QUE. T.	71	132	64	17	APD	F
Mean	71	125	70.2	37.3		
± S.E.M.	0.9	4.3	3.7	11.4		

SBP: systolic blood pressure, DBP: diastolic blood pressure, H: haemodialysis, APD = ambulatory peritoneal dialysis.

Twenty-eight healthy control subjects were studied concomitantly. They were also alloted to three comparable subgroups as to age and sex: group C_1 (mean age: 27.1 + 1.1 yr, mean blood pressure 123.7 ± 3.5/80.0 ± 2.4 mmHg, 5 males, 6 females); group C_2 (mean age: 52.5 ± 1.9 yr, mean blood pressure: 133.0 ± 2.7/74.4 ± 3.1 mmHg, 6 males, 4 females); group C_3 (mean age 78.5 ± 1.5 yr, mean blood pressure: 120 ± 5.2/65.7 ± 4.6 mmHg, 3 males, 4 females). They had taken no medication at least 3 weeks before the study.

All uremic patients and control subjects were investigated between 8 a.m. and 11 a.m. over a period of ten days. The hemodialysed patients were studied just before the dialysis session. After at least a six-hour fast they were confined to bed rest throughout the study and were allowed no smoking or xanthine-containing beverage for twelve hours.

A 19 gauge "butterfly" needle was inserted into a peripheral upper extremity vein (controlateral to the arteriovenous fistulae for the uremic patients) and kept patent with a slow drip of 0.9% saline. After an equilibration period of 60 min, two baseline samples of blood (5 ml) for E and NE were drawn at 10 min interval. Three blood samples were then collected, respectively for PTH (5 ml), total calcium (5 ml) and for MNC separation (40 ml).

Determination of plasma catecholamines

Blood samples were drawn in chilled plastic tubes on lithium heparinate and kept permanently on ice until centrifugation at 4° for 10 min. Plasma was stored at −80° for later determination.

Catecholamines were measured, according to Brown and Jenner [14], by double isotopic enzymatic assay with a sensitivity of 1.5 pg/ml for both E and NE. The coefficients of variation were 3.1% (420 ± 13) and 3.3% (80 ± 2.6) for NE and E respectively (intra-assay) and 4.2% and 4.5% (inter-assay). However, the coefficient of variation for low values of epinephrine was slightly greater, i.e. 10% (25.1 ± 2.5).

Plasma parathyroid hormone

PTH was determined with an anticarboxyl terminal antibody, according to Franchimont *et al.* [15] by radioimmuno-assay with a sensitivity of 1 mUI/ml. The coefficients of variation were 20.2% (17.5 ± 3.5) and 23.6% (1.9 ± 0.4) for high and low values respectively (intra-assay) and 21.9% (10.6 ± 2.2) and 9.4% (3 ± 0.2) (inter-assay).

Radioligand binding assay

Materials. $(-)^{125}$ICYP was supplied by Amersham International and (±) propranolol was obtained from ICI Pharma. A suspending medium (A) containing Tris HCl (25 mM), NaCl (120 mM), KCl (5 mM), $MgCl_2$ (1 mM), $CaCl_2$ (0.6 mM) and glucose (5mM), was adjusted to pH 7.4. A buffer medium (B) containing Tris HCl (50 mM), NaCl (14 mM), KCl (5.4 mM), $CaCl_2$ (1.8 mM) and MgC12 (0.8 mM) was adjusted to pH 7.4 with 1 N HCl at 37° for incubation studies and at 0° for washing the membrane preparations.

Separation of mononuclear cells (MNC). Blood was drawn into syringes containing heparin and centrifuged for 10 min at 20 *g*. The layer of platelet-rich plasma was removed. The lower layer was subjected to Ficoll-Hypaque centrifugation for 40 min at 400 *g* at room temperature, using the method of Böyum [16]. The MNC were recovered at the interphase and washed three times with medium A. Then they were stored at −80° for later determination.

After this storage the burst MNC were homogenized in a Potter-Elvehjem glass homogenizer with three strokes by the motor-driven Teflon pestle. Total protein concentration of the homogenate was determined according to Lowry [17].

Beta-adrenergic receptor binding assay

Binding studies were performed in medium B. MNC preparations were at a final concentration of 0.015 mg protein per ml. Twenty different concentrations of $(-)^{125}$ICYP ranging from 10^{-12} to 8.10^{-11}M, with or without 10^{-6} M propranolol were used in a final volume of 500 µl; The equilibrium was obtained after 75 min at 37°. Reaction was then stopped by immersion of all tubes in ice and samples were rapidly filtered under vacuum through Whatman GF/F glass fiber filters. Each filter was washed twice with 10 ml of medium B used as washing buffer. Radioactivity was determined in a scintillation spectrometer Packard Tricarb 460 CD. Specific binding was defined as total bound radioactivity minus the radioactivity not displaced by 10^{-6} M propranolol. The two parameters, B_{max} and K_d were estimated using a previously published method [18].

Statistical analysis

Comparability of subgroups as to age and sex was checked using the chi_2 test. Results are given as means ± S.E.M. Comparison of control and uremic groups was performed using biological and pharmacological data, by means of two way analysis of variance and Fischer's test. Subgroup means were compared by Student's *t* test based on the residual error term estimated by analysis of variance.

RESULTS

The results are summarized in Table 2.

Under basal conditions all subgroups of uremic patients exhibited significant higher E compared to control subgroups (Fig. 1A). By contrast, NE was not significantly different between uremic and normals, except in the uremic middle-age subgroup (group U_2), which demonstrated significantly higher levels (Fig. 1B).

As expected, the mean value of PTH of each uremic subgroup was significantly higher than in control subjects (Fig. 1C) while total plasma calcium did not differ significantly (data not shown).

As shown in Fig. 2, Scatchard analysis of specific $(-)^{125}$ICYP binding experiment data resulted in linear plots, in control subjects as well as in uremic patients, indicating in both investigated groups the existence of a homogeneous population of beta adrenergic receptors in human lymphocytes. B_{max} was significantly increased in the uremic patients except in the younger subgroup (group U_1) where it

Table 2. Plasma determinations and $(-)^{125}$ICYP binding parameters of each subgroup in both uremic (group U) and control populations (group C)

	C_1 (N = 11)	U_1 (N = 6)	C_2 (N = 10)	U_2 (N = 11)	C_3 (N = 7)	U_3 (N = 8)
E ng/ml*	0.071 ± 0.009	0.136 ± 0.018	0.057 ± 0.006	0.119 ± 0.015	0.049 ± 0.003	0.081 ± 0.006
	$P < 0.001$		$P < 0.001$		$P < 0.05$	
NE ng/ml*	0.551 ± 0.074	0.456 ± 0.056	0.577 ± 0.044	0.883 ± 0.113	0.716 ± 0.141	0.557 ± 0.072
	NS†		$P < 0.01$		NS	
PTH (COOH) mUI/ml*	2.9 ± 0.2	10.1 ± 1.5	3.1 ± 0.2	6.8 ± 0.8	3.5 ± 0.2	7.2 ± 0.4
	$P < 0.001$		$P < 0.001$		$P < 0.001$	
B_{max} fM/mg protein*	15.29 ± 1.87	21.18 ± 4.86	17.56 ± 3.02	27.73 ± 3.11	17.42 ± 2.02	29.12 ± 3.34
	NS†		$P < 0.01$		$P < 0.02$	
K_d pM*	6.6 ± 0.7	7.4 ± 0.7	7.9 ± 0.09	8.8 ± 0.9	9.8 ± 1	8.3 ± 1
	NS†		NS†		NS†	

* Mean ± S.E.M.

† NS: non significant.

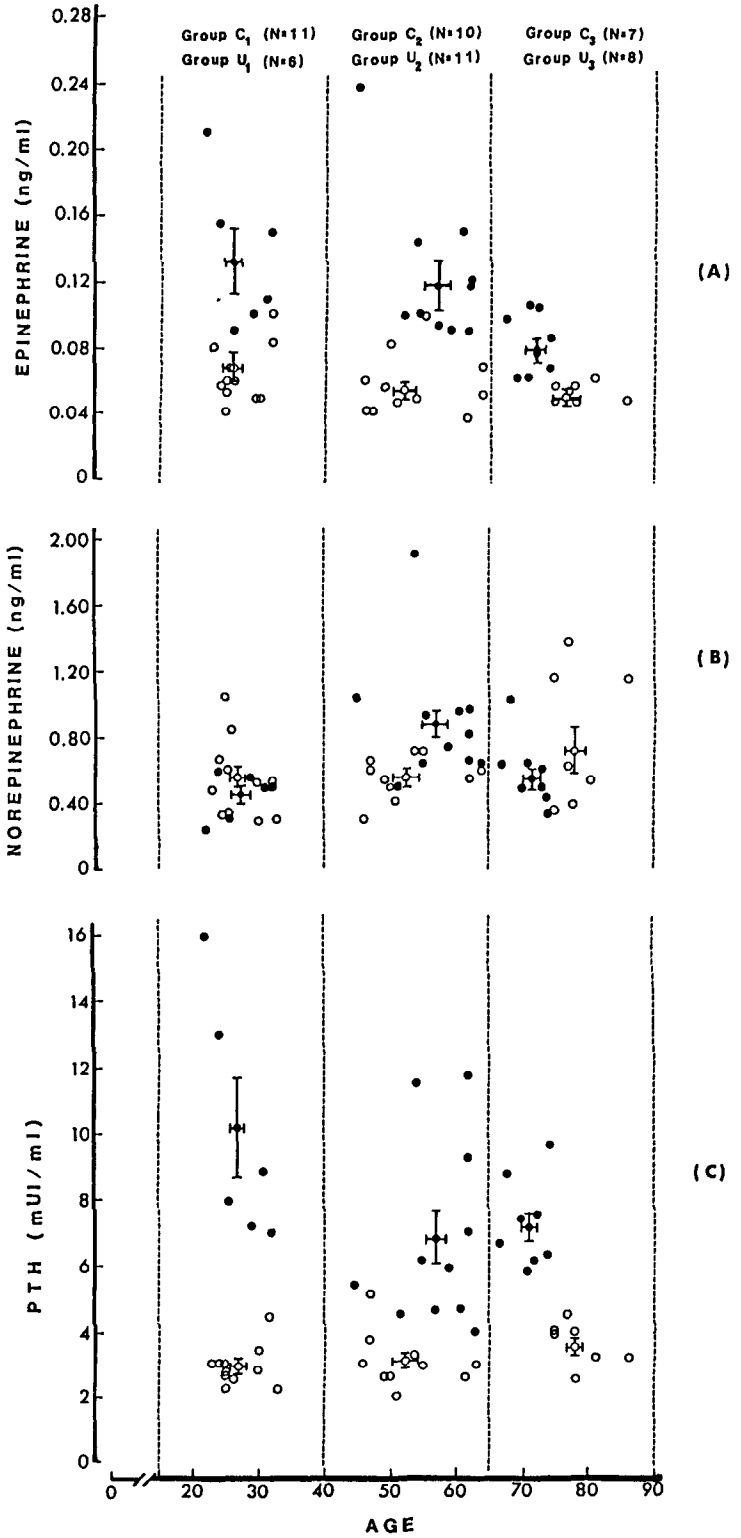

Fig. 1. Comparison of plasma epinephrine (A), norepinephrine (B) and parathyroid hormone (C) in each subgroup of both uremic (●) and control populations (○). Each subgroup value is the mean ± S.E.M.

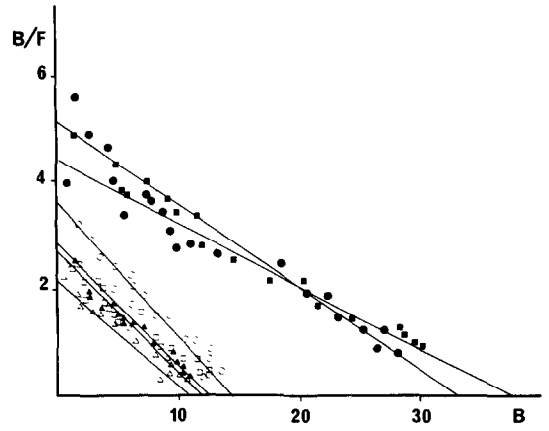

Fig. 2. Scatchard-plot of specific $(-)^{125}$ICYP binding. The ratio B/F of specifically bound $(-)^{125}$ICYP (fmol. mg of protein) to free $(-)^{125}$ICYP (pM) is plotted as function of B: specifically bound $(-)^{125}$ICYP (fmol./mg of protein). Each plot represents the scatchard-plot of one subject chosen in the different subgroups of the control and the uremic patients: △, C_1; ○, C_2; □, C_3; ▲, U_1; ■, U_2; ●, U_3.

did not reach statistical significance (Fig. 3). This increase in B_{max} reflects a rise in the total pool of beta$_2$ receptors in MNC. By contrast, the dissociation constant (K_d) did not differ significantly between uremic and control subjects. Chronic renal disease is therefore associated with both an increase in E and in B_{max} (Fig. 4A). Despite the lack of significant relationships, B_{max} has a tendency to decrease with increasing levels of E in the control group. No significant relationships were found between PTH and B_{max} (Fig. 4B).

On the other hand, in our experimental conditions, E decreases with increasing age in the control and in the uremic groups. In addition in the control group there is a tendency of NE to increase with age. Nevertheless, age had no significant influence on these parameters. In the same way age did not alter the binding parameters of $(-)^{125}$ICYP in both groups.

DISCUSSION

It has been reported that plasma catecholamines are elevated in patients undergoing chronic hemodialysis [19–21]. In our study, E in the group of uremic patients was significantly higher whereas NE was not significantly different compared to the values observed in control subjects, except in one subgroup. These results are in contrast with a previous work which showed lower catecholamine levels under similar conditions [22]. The differences in methodologies of the assays used by the various laboratories and probably other factors may account for these differences. In our study, all the uremic patients were normotensive and were age and sex-matched.

In our study, age did not significantly influence catecholamine levels or beta$_2$-adrenoceptor density. In the literature, these two parameters are described as respectively slightly increased or unchanged with age [23–25].

Data from the literature show that an increase in plasma catecholamines is associated with a decrease of adrenoceptor density, a phenomenon called "downregulation" [13]. Previous works have reported that the number of beta$_1$ and beta$_2$

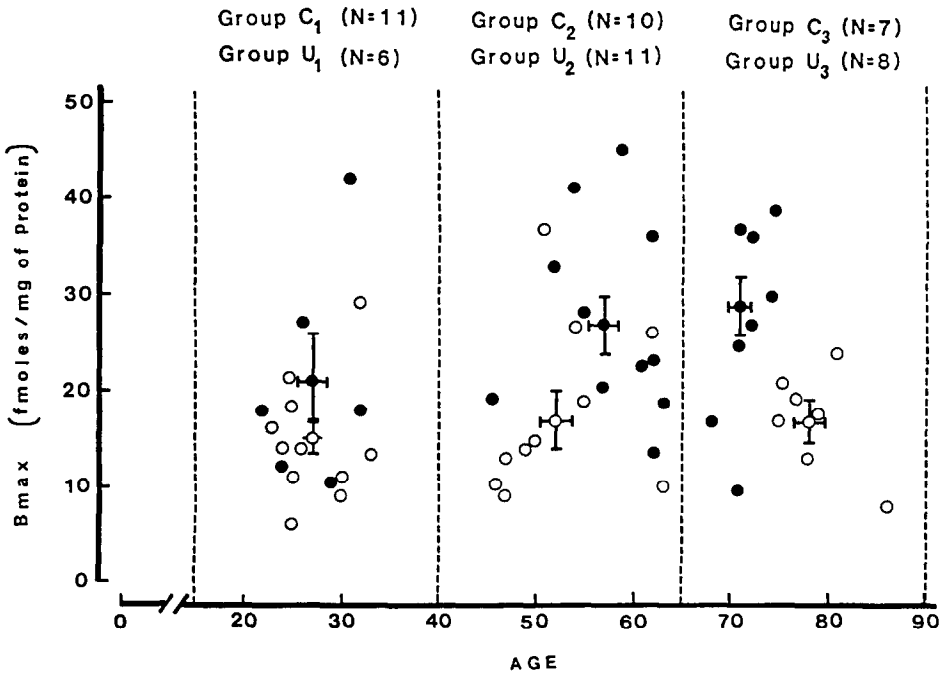

Fig. 3. Comparison of B_{max} of each subgroup in both uremic (●) and control (○) populations. Each subgroup value is the mean ± S.E.M.

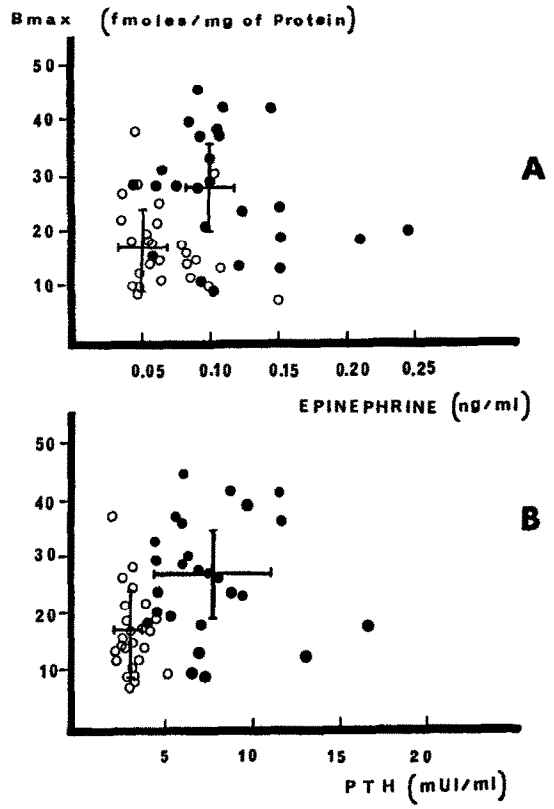

Fig. 4. (A) Relationship between B_{max} and plasma epinephrine. (B) Relationship between B_{max} and parathyroid hormoneuremic subjects (●), control subjects (○), (mean ± S.E.M.).

adrenergic receptors may be independently regulated by endogenous catecholamines. Although a significant relationship has not been shown between leukocyte beta$_2$ adrenergic receptors density and circulating levels of E and NE in normotensive subjects [13] other studies have demonstrated that pathophysiological levels of NE down regulated the beta$_1$ and not the beta$_2$ subtype [26–28]. This implies that E has a more pronounced regulatory effect on the beta$_2$ subtype. Despite elevated E, we observed a significant and unexpected increase in beta$_2$-adrenoceptors in the uremic patients. These facts suggest that the downregulation in beta$_2$-adrenoceptors is impaired in uremia, possibly due to an altered relationship between catecholamines and adrenergic receptors.

Furthermore uremia is characterized by a decrease in beta adrenoceptor mediated responsiveness [3–5], suggesting a decrease in beta-adrenoceptor function. Cyclic AMP levels in lymphocytes in response to isoprenaline stimulation is diminished in patients on maintenance hemodialysis [29]. Therefore it is possible that a uremic toxin modulates the number and response of beta-adrenergic receptors and that this toxin impairs the downregulation of the beta receptors by catecholamines. Accordingly, human subjects treated by beta antagonists such as propranolol demonstrated mostly upregulation, leading to an increase in beta-adrenoceptor density [8, 30, 31].

The elevated E in the uremic state contrasts with a surprising upregulation of beta-adrenoceptors, an interaction comparable to what is observed during beta-blocking treatment. On the other hand beta antagonists have been demonstrated to increase both E and NE in normal volunteers [32], a phenomenon due to a decrease of catecholamines total body catecholamines clearance by 60% [33], suggesting a comparable situation in terms of catecholamines and beta-adrenoceptor upregulation between uremia and beta blockade in man. Therefore, the role of an endogeneous beta-blocking agent during chronic renal failure is suggested.

Although beta$_2$-receptor density is increased, the dissociation constant of $(-)^{125}$ICYP is not impaired.

The uremic toxin thought to interfere with the beta-adrenergic response has not yet been clearly identified. Previous studies, however, tend to prove that PTH might play a role either indirectly by producing metabolic changes, or directly via an effect on beta-adrenoceptors. In fact, *in vitro* studies have demonstrated that the depressant effect of propranolol on myocardial contractile force was significantly inhibited by ultrafiltrates of uremic patients with severe hyperparathyroidism [4, 5]. Moreover, synthetic (1–34) and (1–84) PTH reduced the effect of beta agonists or antagonists and induced a positive dose-dependent chronotropic effect on cultured rat myocardial cells [4, 34–36], suggesting an intrinsic sympathomimetic activity. In uremic patients, the hyperparathyroid state is associated with an altered heart-rate responsiveness to isoproterenol [3] and parathyroidectomy is followed by a significant improvement in left ventricular ejection fraction [37].

None of our uremic patients developed severe secondary hyperparathyroidism, but all of them had a significant increase in parathyroid hormone. However, the absence of a significant correlation between B_{max} and PTH suggested that other factors might be involved in the altered regulation of beta$_2$-receptors during chronic renal failure.

From our data, it can be concluded that the uremic state is associated with an impaired regulation of beta$_2$-receptor density in MNC.

If receptor density measured on human MNC reflects receptor density in other tissues [38], our findings contribute to the understanding of altered adrenergic responsiveness during chronic renal failure.

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REFERENCES

1. J. J. Lilley, J. Golden and R. A. Stone, *J. clin. Invest.* **57**, 1190 (1976).
2. E. S. Kersh, S. J. Kronfield, A. Unger, R. W. Popper, S. Cantor and K. Cohn, *N. Engl. J. Med.* **290**, 650 (1974).

3. A. Ulmann, T. Drüeke, J. Zingraff and J. Crosnier, *Clin. Nephrol.* **7**, 58 (1977).
4. T. Drüeke, F. Lhoste, S. Larno, J. Zingraff, N. K. Man, P. Jungers, J. R. Boissier and J. Crosnier, *Proc. Eur. Dial. Transpl. Ass.* **13**, 464 (1976).
5. F. Lhoste, T. Drüeke, S. Larno, N. K. Man, J. Zingraff, P. Jungers and J. R. Boissier, *Biomedecine* **25**, 181 (1976).
6. H. J. Motulsky and P. A. Insel, *N. Engl. J. Med.* **307**, 18 (1982).
7. L. T. Williams, R. Snyderman and R. J. Lefkowitz, *J. clin. Invest.* **57**, 149 (1976).
8. R. D. Aarons, A. S. Nies, J. Gal, L. R. Hegstrand and P. B. Molinoff, *J. clin. Invest.* **65**, 949 (1980).
9. O. E. Brodde, G. Engel, D. Hoyer, H. D. Bock and F. Weber, *Life Sci.* **29**, 2189 (1981).
10. R. M. A. Landmann, E. Bürgisser, M. Wesp and F. R. Bühler *J. Rec. Res.* **4**, 37 (1984).
11. A. Van Es, H. J. Tanke, W. M. Balwin, P. J. Oljans, J. S. Ploem and L. A. Van Es, *Clin. exp. Immunol.* **52**, 13 (1983).
12. M. Fiche, J. P. Soulillou, J. D. Bignon, S. Billaudel and J. Guenel, *Transplantation* **37**, 421 (1984).
13. J. Fraser, J. Nadeau, D. Robertson and A. J. J. Wood, *J. clin. Invest.* **67**, 1777 (1981).
14. M. J. Brown and D. A. Jenner, *Clin. Sci.* **61**, 591 (1981).
15. P. Franchimont and G. Heynen, in *Parathormone and Calcitonine Radioimmunoassay*. Masson, Paris, (1976).
16. A. Böyum, *Scand. J. clin. Lab. Invest.* **21**, (Suppl. 97), 77 (1968).
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall *J. biol. Chem.* **113**, 265 (1951).
18. R. Zini, I. Gault, S. Ledewyn, P. D'Athis, J. P. Tillement, *Biochem. Pharmac.* **32**, 3375 (1983).
19. N. O. Atuk, C. J. Bailey, S. Turner, M. Peach and F. Westervelt, *Trans. Am. Soc. Artif. Internal Organs* **22**, 195 (1976).
20. J. L. Izzo and R. H. Merns, *Kidney Int.* **24**, (Suppl. 16), s221 (1983).
21. H. M. Brecht, W. Ernst and K. M. Koch, *Proc. Eur. Dial. Transplant Assoc.* **12**, 281 (1975).
22. C. N. Corder, J. Sharma and R. H. McDonald, *Nephron.* **25**, 267 (1980).
23. M. G. Ziegler, C. R. Lake and I. J. Kopin, *Nature, Lond.* **261**, 333 (1976).
24. V. Doyle, K. O'Malley and J. G. Kelly, *J. Cardiovascul. Pharmac.* **4**, 738 (1982).
25. R. Landmann, H. Bittiger and F. R. Bühler, *Life Sci.* **29**, 1761 (1981).
26. O. E. Brodde, M. Anlauf, N. Graben and K. D. Bock, *Eur. J. Pharmac.* **81**, 345 (1982).
27. G. Tsujimoto, W. M. Manger and B. B. Hoffman, *Endocrinology* **114**, 1272 (1984).
28. M. D. Snavely, H. J. Motulsky, E. Moustafa, L. C. Mahan and P. A. Insel, *Circ. Res.* **51**, 504 (1982).
29. O. E. Brodde, and A. Daul, *Contr. Nephrol.* **41**, 99 (1984).
30. O. E. Brodde, A. Daul, N. Stuka, N. O'Hara and U. Borchard, *Naunyn Schmiedebergs. Arch. Pharmac.* **328**, 417 (1985).
31. Y. Giudicelli, D. Lacasa, B. Agli and A. Leneveu, *Eur. J. clin. Pharmac.* **26**, 7 (1984).
32. C. Gennari, G. Pollavini, R. Nami, G. Francini, C. Bianchini and P. Verdecchia, *Eur. J. clin. Pharmac.* **26**, 695 (1984).
33. P. E. Cryer, R. A. Rizza, M. W. Haymond and J. E. Gerich, *Metabolism* **29**, (Suppl. 1), 1114 (1980).
34. S. Larno, F. Lhoste, M. C. Auclair and P. Lechat, *J. Mol. Cell. Cardiol.* **12**, 955 (1980).
35. F. Lhoste, T. Drüecke, S. Larno and J. R. Boissier, *Clin. exp. Pharmac. Physiol.* **7**, 119 (1980).
36. E. Bogin, S. G. Massry and I. Harary, *J. clin. Invest.* **67**, 1215 (1981).
37. T. Drüeke, J. Fleury, Y. Toure, P. De Vernejoul, M. Fauchet, P. Lesourd, C. Le Pailleur and J. Crosnier, *Lancet* **ii**, 112 (1980).
38. R. D. Aarons and P. B. Molinoff, *J. Pharmac. exp. Ther.* **221**, 439 (1982).